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# The Impact of Gelrite and Activated Carbon on the Elemental Composition of Plant Tissue Culture Media

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## Abstract

The elemental composition of plant tissue culture media was studied in response to (a) different levels of Gelrite and activated carbon in semisolid media, and (b) different levels and types of activated carbon in liquid media. Doubling Gelrite from 2 to 4 g/L, reduced available magnesium (20%), zinc (17%) and manganese (24%) and increased potassium (6%). Activated carbon adsorbed calcium from semisolid medium (25%). Copper (90-95%) and zinc (35-51%) were adsorbed from both liquid and semisolid media. Two significantly different ACs gave minor differences in adsorption. No adsorption was indicated for inorganic anions. Non-acid-washed activated carbon released significant levels of magnesium (44%

increase), calcium (16% increase) and silica (75% increase to 1.8 ppm). Elemental composition of media may need to be adjusted when increasing the Gelrite level or adding activated carbon.

*Key words: tissue culture activated carbon elemental composition Gelrite*

# Introduction

Several common components of tissue culture media are of biological or geological origin with variable chemical composition including coconut milk, casein hydrolysate, agar, phytagel or gellan gum (Gelrite), and activated carbon (AC).

Agar, a seaweed extract, is reportedly more variable and chemically less pure than gellan gum (Bonga 1992), a bacterial product. Both are primarily composed of polysaccharides. Improvements in culture success have been reported when using gellan gum (Tremblay 1991). Gelrite is known to bind cations, particularly $Ca^{2+}$ and $Mg^{2+}$. It also has a high ash content relative to tissue-culture grades of agar; therefore, it may introduce inorganic impurities into the growth medium (Scherer et al. 1988).

AC is often added to plant tissue culture medium with improved culture success. Pan and van Staden (1998) recently reviewed the use of charcoal in vitro. AC was originally added to media to darken the medium and simulate soil conditions (Proskauer, Berman 1970). Johansson and Eriksson (1977) found that AC improved anther culture in a semisolid medium, results were later confirmed using a double-layer medium (Johansson et al. 1982). Kohlenbach and Wernicke (1978) reported that AC removed inhibition due to agar when culturing anthers. When used in a double-layer medium with increased calcium levels, AC improved embryogenesis in cucumber (Ziv, Gadasi 1986). AC was found to improve embryogenesis in microspore cultures (Guo, Pulli 1996). Detrimental effects have also been reported when including AC in growth media. These include inhibition of root formation (Menzuali-Sodi 1986) and inhibition due to adsorption of various medium components (Zaghmout, Torello 1988, George and Sherington 1984).

Improvements in success when adding AC to the growth medium have been attributed to different factors. The adsorption of various organic compounds has been reported. These compounds include excess hormones (Constantin et al. 1977, Weatherhead et al. 1978, Johansson et al. 1982, Nissen, Sutter 1990), vitamins (Weatherhead et al. 1979), abscisic acid (Johansson et al. 1982, Pullman, Gupta 1991), phenolic metabolites (Weatherhead et al. 1979) and ethylene from the growing culture (Menzuali-Sodi et al. 1993). Additionally, adsorption of 5-hydroxymethyl furfural, an inhibitory byproduct of autoclaving sucrose, has been reported (Weatherhead et al. 1978).

The impact of AC on the inorganic composition of the medium has received less attention. The possible release of ionic material was investigated by Weatherhead et al. (1979). The adsorption of iron chelates in culture media has also been indicated (Haberle-Bors 1980). Literature from other disciplines indicate that AC adsorbs many different inorganic species. Sorptive responses have been reported for $Cu^{+2}$, $Pb^{+2}$ (Wilcszak, Keinath 1993), $Co^{+2}$ and $Fe^{+3}$ (Howard 1988), $Zn^{+2}$ (Gambaldon et al. 1996), $Ni^{+2}$ (Reed, Nonavinakere 1992, Corapcioglu, Huang 1987), chromium ions (Bautista-Toledo et al. 1994), $Ca^{+2}$, silver-, gold-, and mercury-cyanide ions (Kongolo et al. 1997, Petersen, van Deventer 1997), and others (Qadeer et al. 1993, Hall, Pelchat 1993). Adsorption of ligated species such as Cu-EDTA (Chang, Ku 1995), and Cd-EDTA (Rubin, Mercer 1987, Chang, Ku 1994) have also been reported.

This paper considers the impact of different Gelrite levels, AC type, and the combination of Gelrite and AC on the mineral nutrient composition of plant growth media. Preliminary reports on part of this work have appeared (Pullman et al. 1995, Van Winkle, Pullman 1997).

# Experimental

## Materials

The media were formulated from tissue culture-grade reagents supplied through Sigma, with the exception of casamino acids (Difco). Media were formulated using Nanopure deionized water.

Two different grades of AC were used. These were supplied by Sigma as untreated powder (C-5260), designated "N" type, and acid-washed tissue culture tested powder (C-9157), designated "T" type. Two production lots, designated "1" and "2" for each type, were characterized.

## Activated Carbon Characterization

Ash % was determined on dry AC (dried in a vacuum oven at ca. 120°C overnight) through thermogravimetric analysis with temperature of 650°C. Ash samples were carbon coated and analyzed qualitatively for their metal composition using x-ray dispersive analysis, Jeol SEM (JSM 6400 with Link eXL detector). The point of zero charge (pzc) was approximated by mass titration, a technique that involves adding an increasing mass of AC to a given mass of water until some limiting pH value is approached. The apparent surface area (BET) was determined by nitrogen adsorption (Micromeretics Flowsorb II 2300).

## Media Preparation

The semisolid medium (Table 1) was a modified formulation for loblolly pine culture based on ½ P6 basal salts (Teasdale et al. 1988) and pH adjusted to 5.2. The liquid media (Table 1) was based on the media for Norway spruce culture reported by Verhagan and Wann (1989), a modified half-strength Brown and Lawrence (BLG) formulation (Amerson et al. 1985) with pH adjusted to 5.8.

Media were formulated, pH adjusted with 0.1M KOH, and autoclaved for 20 minutes at 121°C. Filter-sterilized glutamine was added after autoclaving. For the experiments concerning the effect of EDTA, media were formulated without Fe-EDTA but with otherwise complete media, and in a separate treatment, media were formulated with only the mineral nutrients, including Fe-EDTA.

The media formulations are presented in Table 1.

**Table 1.**

## Elemental Analyses

Elemental analyses were performed on complete and partial media using inductively coupled plasma atomic emission spectroscopy (ICP-AES, Perkin Elmer Optima 3000DV). For the semisolid media, the low levels of copper, cobalt, and boron were analyzed using atomic absorption by Analytical & Research Laboratories, Atlanta.

For the semisolid media, liquid (100 mL) was extracted from the gel by vacuum filtration through a 0.45-μm filter. Samples were collected after equilibrating ten days. Liquid media reached equilibrium faster, samples were collected after two days by passing media (10 mL) through a rinsed syringe filter (Gelman Acrodisc 0.2 μm, HT Tuffryn® membrane) and discarding the initial 2 mL. Samples were acidified with two drops of nitric acid prior to ICP analysis. The experimental data were the result of three or more replications, except as noted, and instrumental measurements of each sample were repeated in triplicate. Data for liquid media were obtained over a pH range of 5.6 to 5.9 with the exception of selected ions, for which the data were pooled across a pH range from 4.8 to 6.0. All of the data for semisolid media were collected from media adjusted to an initial pH of 5.2.

The chloride and nitrate levels for liquid media were determined using capillary ion electrophoresis (Water Capillary Ion Analyzer) with fused silica capillaries and a high-mobility electrolyte.

## Results and Discussion

The carbon characterization data appear in Table 2. It may be seen that the four ACs were basic in nature, with the untreated, N-type carbon more basic than the T-type. Production lot variation was pronounced. The ash content for N-type AC was significantly higher than that for T-type. The ash proved to be only partially soluble in common acids (acetic, hydrochloric, nitric) and was therefore qualitatively analyzed by x-ray dispersive analysis for metal atoms, SEM-EDX. This analysis revealed similar elements, Mg, Al, Si, P, S, Na, K, Ca, and Fe for both carbons, suggesting the presence of clays and sulfates. The relative peak heights for Mg and Ca indicated significant differences in atomic percentage composition for the two types of ACs. The differences in total ash content were attributed to acid-washing of tissue culture-grade AC, which is believed to result in the dissolution of a portion of the inorganic contaminants. Despite acid washing, T-type AC retained a significant ash level. The BET data in Table 2 indicate that the four carbons were significantly different in surface area, but all were in good agreement with carbons used for water treatment.

**Table 2.**

The data for semisolid media resulted from a two-by-two factorial experiment involving two different levels of Gelrite (2 g/L and 4 g/L) and two different levels of AC (0 and 2.5 g/L). The data for liquid media compared the effect of two different ACs (T1 and N1) with a control (liquid media without AC). Since the semisolid and liquid media were for two different species, the calculated levels for most ions were different (refer to Table 1). The evaporative loss of volume due to autoclaving was measured as 4.5% on average. The following figures include the autoclave-adjusted calculated target levels as reference for the different treatments.

Elemental data (ICP) for the macronutrients, potassium and calcium, are presented in Figure 1. For each element the data exceeded the calculated levels. The elevated potassium levels, relative to the target, were attributed primarily to the use of KOH as a base for dissolving acidic components and for pH adjustment, and to impurities introduced with the medium components. The semisolid data also revealed elevated potassium when AC was present, a reflection of higher 2,4-D levels, which resulted in the addition of more KOH. Additional potassium was introduced with the casamino acids, approximately 1.4 ppm, and the Gelrite, ca. 28 mg/g (Scherer et al. 1988). The potential increase in potassium in the gelled medium due to Gelrite was therefore 56 or 112 ppm for media with 2 or 4 g/L, respectively. The actual increases in potassium for gelled media were 73 and 98 ppm. In comparison, the liquid media displayed similar potassium levels for all three treatments, indicating that the increase in potassium for semisolid media with AC was not due to the carbon.

**Figure 1.**

The calcium data for semisolid media gave inexplicably high levels and were quite variable. Based on previous studies that showed Gelrite to bind calcium, the higher levels of calcium could not be attributed to Gelrite (potential total contribution of ca. 5 mg/g, Scherer et al. 1988). The effect of AC was a statistically significant reduction in calcium (25% on average) for each level of Gelrite. For liquid media, the calcium level increased slightly when using non-acid-washed AC, N1, but was not different from the control when using tissue culture-grade carbon, T1. This effect may be attributed to the high ash levels in the non-acid-washed carbon; i.e., calcium was released.

Data for sodium are presented in Figure 2. Sodium levels were similar for semisolid and liquid media but were much higher than the calculated levels for all treatments. The elevated sodium levels were due to the casamino acids, which are 13.7% sodium by mass (according to manufacturer's elemental analysis), and Gelrite, which may potentially add

an additional 6.8 mg/g. The total amount of sodium present, ca. 70 ppm, was in good agreement with the contribution from casamino acids alone.

**Figure 2.**

Data for magnesium are presented in Figure 3. It may be seen that magnesium levels, in general, were close to calculated levels. The lower level of available magnesium with increasing Gelrite was consistent with the sequestering of Mg attributed to Gelrite. AC (T1) had no impact on magnesium availability in gelled media. For liquid media, T1 had no impact on magnesium compared to the control, whereas media with N1 displayed significantly higher magnesium levels. As with the increase in calcium, this increase was attributed to the high ash content in the non-acid-washed carbon.

**Figure 3.**

Results for manganese and iron are presented in Figure 4. The manganese levels were significantly higher than calculated for the semisolid media but could not be attributed to the gelling agent, as doubling the Gelrite resulted in a 24% reduction on average. Addition of AC to semisolid media resulted in lower manganese levels (-7%). The liquid media showed no effect on manganese due to AC. Iron levels were lower than calculated for all of the treatments, increasing slightly with increased Gelrite or in N1 treatments. Scherer et al. (1988) reported Gelite iron content to be 0.28 mg/g.

**Figure 4.**

Data for zinc and boron are depicted in Figure 5. Gelrite had a significant impact on zinc levels, resulting in a decrease (ca. 50%) from the calculated level, which may be attributed to sequestering. Of the remaining zinc, 51% was depleted on average when AC was included in the medium. As a result of this finding, the initial level of zinc in liquid media was doubled to allow for adsorption. Subsequent adsorption of zinc resulted in available zinc levels near the desired target for each AC. The boron level was significantly lower than calculated for media with Gelrite, suggesting a reduction in

solubility. For liquid media, the measured boron levels were higher than the calculated level. AC had no effect on boron levels in either semisolid or liquid media.

**Figure 5.**

Data for copper are presented in Figure 6. The effect of Gelrite was variable, but may contribute copper to the medium. AC adsorbed 90% of the copper present in semisolid media. As a result of this finding, the initial level of copper in liquid media was raised 20 times to compensate for adsorption. In liquid media 93-95% of the copper was adsorbed onto AC. Though copper levels were near the calculated target level for liquid media with each carbon type, slightly more adsorption onto T1 occurred resulting in less available copper than was present for media with N1. The measured levels of copper and zinc adsorption were sufficient to lead to deficiency symptoms in media with low initial levels of these elements. These findings suggest that copper and zinc may require adjustment in media containing AC, especially when levels of AC are high, basal media contain low levels of copper or zinc, or when plant tissue requires high levels of these micronutrients.

**Figure 6.**

Cobalt data were collected for both media, but proved to be too variable to draw any conclusions. The adsorption of cobalt by AC has been noted in other literature (Howard 1988), however.

For nonautoclaved medium, the rate of adsorption of copper was quite rapid with over 70% adsorbed within half an hour of mixing the medium (Figure 7). The adsorption continued to increase over the next two hours. Autoclaving appeared to accelerate equilibration with only slightly lower levels measured after 12 days. Zinc data were similar (not shown). Depletion (precipitation) of iron was also promoted by autoclaving. Copper adsorption increased with autoclaving in agreement with an endothermic process. Ferro-Garcia et al. (1988) reported the adsorption of coppor onto AC as endothermic and zinc as exothermic, indicating different adsorption mechanisms.

## Figure 7.

Though adsorption of Fe-EDTA was insignificant for complete media, it has been shown to adsorb to AC in other systems (Xue at al. 1995). Additionally, Cu-EDTA was found to adsorb to AC (Chang, Ku 1995). Omitting the organic components from media with AC resulted in significant removal of the available iron and zinc (Figure 8). Spectroscopic data (UV) confirmed that EDTA was also removed to a level below the detection threshold of the instrument. The lack of adsorption of Fe-EDTA in complete media was attributed to competitive sorption from less-soluble organic components of the medium, primarily 2,4-D. Zinc adsorption increased with omission of the organic components, resulting in a decrease in available zinc of about 25%. The omission of Fe-EDTA and the other organic medium components resulted in a further increase in zinc adsorption, whereas copper adsorption increased by only a few percentage points. Omitting the other organic components from the medium while adding Fe-EDTA led to reduced copper adsorption, resulting in an increase in available copper level by a factor of 3.7. Ferro-Garcia at al. (1988) reported that the addition of EDTA reduced adsorption, and other complexing agents actually increased the adsorption of copper and zinc. These results indicate that copper and zinc adsorbed as hydrated inorganic compounds and also, possibly, as organic complexes. The difference in response for copper and zinc suggests that they adsorbed to different sites on the surface of the carbon.

## Figure 8.

A plausible explanation for zinc adsorption may be found in the mechanism that has been proposed to account for the precipitation phenomena observed in Murishige and Skoog tissue culture media (Teasdale 1987). The central premise is that the iron, $Fe^{3+}$, precipitates as phosphate (Dalton 1983). The decline in iron results in free EDTA to chelate other metals: $Cu^{2+}$, $Zn^{2+}$, $Ni^{2+}$, $Co^{2+}$, $Fe^{2+}$, and $Mn^{2+}$, in agreement with the Irving-Williams sequence of decreasing complex stability (Morel, Hering 1993). The

decline in available zinc was consistent with the mechanism proposed by Teasdale: omitting complex-forming organic compounds, including EDTA, resulted in increased adsorption of zinc.

However, when the organic components, with the exception of Fe-EDTA, were omitted, copper adsorption dropped from 93-95% to about 80%, resulting in a significant, nearly fourfold increase in available copper. Omitting Fe-EDTA and other organics, treatment in Figure 8 labeled "No Fe-EDTA" resulted in normal adsorption of copper. According to the mechanism proposed by Teasdale, the omission of Fe-EDTA from the medium should have resulted in significantly reduced adsorption of copper, as copper is predicted to be completely chelated, followed by zinc and then manganese. This result indicates that copper adsorbed independently of EDTA.

Some minerals are carried into tissue culture media as impurities of added components. Data for nickel and aluminum were highly variable and thus inconclusive. Aluminum ranged up to ca. 0.025 ppm in liquid media and nickel was present up to 0.14 ppm in gelled media with AC, but less than 0.002 ppm in liquid media, when present. Liquid media data were also collected for other elements: Cr, Si, Se, As, Ag, Cd, Sn, Sb, Pb, Tl, Sr, Ba, Be, Ti, and V. Of these, only Si, Se, Sr, and Ba were present at levels above detection (Table 3). Silicon and strontium increased when AC was included.

**Table 3.**

Though ICP does not directly measure anions, it does produce data for cations that result when anions dissociate in the argon flame within the instrument. Thus, data were collected for sulfur (sulfate), phosphorus (phosphate), and molybdenum. Additional data for anions in liquid media were collected for nitrate and chloride using capillary ion electrophoresis. These data are presented in Table 4.

**Table 4.**

Referring to Table 4, molybdenum was present at approximately 40% less than the calculated value for each treatment. Similarly, the measured level of chloride was significantly lower (25%) than the calculated value. (Note that the calculated value for chloride includes the chloride expected to be added with casamino acids, 21.2 % chloride by mass, or 105 ppm Cl per 500 mg casamino acids). The low level of molybdate and chloride in the control, however, indicated that adsorption onto AC was not responsible. Precipitation may account for the loss of molybdenum but is less likely in the case of chloride. As the capillary electrophoresis instrument was not calibrated against other chlorine-containing species, it is possible that a portion of the chlorine was present in a different form and not accounted for. The data in Table 4 indicate that, in general, the inorganic anions in the medium were not adsorbed onto AC. However, adsorption of organic anions (dissociated organic acids) is well documented in the AC literature (Mattson and Mark 1971) and the adsorption of 2,4-D has been noted previously (Mattson and Mark 1971, Ebert and Taylor 1990, Ebert et al. 1993). Watson et al. (1973) reported that chloride and by extension the other anions may be out-competed by 2,4-D. That finding was consistent with the adsorption of Fe-EDTA noted previously.

## Conclusions

In summary, the increased addition of Gelrite to media (from 2 g/L to 4 g/L ) resulted in relatively minor changes in the ionic composition of the medium; the largest change resulted from binding of magnesium (ca. 20% loss), a reduction in available zinc (17% loss), and a reduction in available manganese (24%). For semisolid media, AC adsorbed copper, zinc (90+ and 51%, respectively), calcium (25%), and manganese (7%). In liquid media, only adsorption of copper and zinc was observed with adsorption varying slightly with carbon type, Cu adsorption of 93 and 95% for non-acid-washed (N1) and tissue culture grades (T1), respectively, and Zn adsorption of 35 and 46%. The difference in response between the semisolid and liquid media was attributed to different relative concentrations of mineral components and a higher level of AC in semisolid medium.

Compensating for carbon adsorption by doubling zinc and increasing copper by a factor of twenty resulted in residual levels that were near the calculated target levels. Copper and zinc adsorption occurred quite rapidly, with the bulk occurring before the media were autoclaved. Autoclaving, in general, resulted in more rapid equilibration for both precipitation and adsorption phenomena.

The release of ionic material from AC was a more significant factor for a non-acid-washed type as opposed to tissue culture-grade AC, resulting in significant increases in calcium and magnesium (18 and 45%, respectively). The higher levels of release were attributed to higher soluble ash content in the non-acid-washed carbons. High levels of sodium (ca. 70 ppm) were primarily attributed to technical-grade casamino acids.

Chelated iron was normally not adsorbed onto AC. However, when the other organic medium components were omitted, more than 95% of the chelated iron was adsorbed, indicating that competition from organic compounds determined the level of adsorption for chelated species.

These results indicate that both Gelrite and AC have an impact on the ionic composition of the medium, which may help to explain some of the variation in culture success reported in the literature.

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Table 1a. Comparison of semisolid and liquid culture media formulations, mineral components, ppm.

| Media | # 201 | # 403 |
|---|---|---|
| | Semisolid [a] | Liquid [b] |
| $NH_4NO_3$ | 200 | |
| $KNO_3$ | 909.9 | 50 |
| $KH_2PO_4$ | 136.1 | 85 |
| KCl | | 372.5 |
| $CaCl_2 * 2H_2O$ | | 220 |
| $Ca(NO_3)_2*4H_2O$ | 236.2 | |
| $MgSO_4 * 7H_2O$ | 246.5 | 160 |
| $Mg(NO_3)_2*6H_2O$ | 256.5 | |
| $MgCl_2*6H_2O$ | 101.7 | |
| KI | 4.15 | 0.415 |
| $H_3BO_3$ | 15.5 | 3.1 |
| $MnSO_4 * H_2O$ | 10.5 | 8.45 |
| $ZnSO_4 * 7H_2O$ | 14.4 | 4.3;8.6 |
| $Na_2MoO_4 * 2H_2O$ | 0.125 | 0.125 |
| $CuSO_4 * 5H_2O$ | 0.125 | 0.0125; 0.25 |
| $CoCl_2 * 6H_2O$ | 0.125 | 0.0125 |
| $FeSO_4 * 7H_2O$ | 13.9 | 13.9 |
| $Na_2EDTA$ | 18.65 | 18.65 |

[a] Semisolid: Modified ½ P6 (Teasedale, Woolhouse 1988)

[b] Liquid: Modified ½ BLG (Verhagen, Wann 1989)

Table 1b. Comparison of semi-solid and liquid culture media formulations, organic components, ppm.

| Media | # 201 | # 403 |
|---|---|---|
| | Semisolid [a] | Liquid [b] |
| Maltose | 15,000 | |
| Sucrose | | 10,000 |
| Myo-Inositol | 20,000 | 50 |
| Casamino acids | 500 | 500 |
| L-Glutamine | 450 | 750 |
| Thiamine HCl | 1 | 0.05;0.15 |
| Pyridoxine HCl | 0.5 | 0.05;0.15 |
| Nicotinic acid | 0.5 | 0.25;0.75 |
| L-Asparagine | | 50 |
| Glycine | 2 | |
| 2,4-D | 1.1;220 | 2;100 |
| BAP | 0.45;90 | 1;90 |
| Kinetin | 0.43;86 | |
| Activated carbon | 0;2500 | 0;1250 |
| Gelrite | 2000;4000 | |
| PH | 5.2 | 5.8 |

[a] Semisolid: Modified ½ P6 (Teasedale, Woolhouse 1988)

[b] Liquid: Modified ½ BLG (Verhagen, Wann 1989)

1 Table 2. Summary of physical and chemical character of activated carbon.

| | Ash (%) | PZC (pH) [b] | BET ($m^2/g$) [c] |
|---|---|---|---|
| T1 [a] | 2.8 | 8.5 | 1050 |
| T2 | 2.6 | 10 | 940 |
| N1 | 5.8 | 10.7 | 560 |
| N2 | 8.7 | 11 | 710 |

2 [a] T designates tissue culture grade; N designates nontreated activated carbon.

3 1 and 2 designate different production lots.

4 [b] PZC is the point of zero charge.

5 [c] BET is the Brunauer Emmett and Teller method applied to nitrogen adsorption data.

1 Table 3. Elements (ppm) present in liquid media as impurities; activated carbon effect.

| | Control | | T1 | | N1 | |
|---|---|---|---|---|---|---|
| | Avg | Stdev | Avg | Stdev | Avg | Stdev |
| Si | 1.07 | 0.0424 | 1.34 | 0.062 | 1.88 | 0.077 |
| Se | 0.038 | 0.0007 | 0.038 | 0.0053 | 0.041 | 0.012 |
| Sr | 0.031 | 0.0005 | 0.032 | 0.0005 | 0.072 | 0.0067 |
| Ba | 0.013 | 0.0120 | 0.022 | 0.0008 | 0.025 | 0.0026 |

2

Table 4. Anionic composition of liquid media, ppm.

| | Calculated | Control [a] | T1 | N1 |
|---|---|---|---|---|
| S | 25.8 | 26.9 | 26.6 | 27.5 |
| P | 20.2 | 21.4 | 21.9 | 20.6 |
| Mo | 0.05 | 0.029 | 0.029 | 0.03 |
| $Cl^-$ | 422 [b] | 294 | 293 | n/a [c] |
| $NO_3^-$ | 32.0 | 26.5 | 27.3 | n/a |

[a] With the exception of AC, identical in composition to AC media.

[b] Includes expected contribution due to casamino acids.

[c] n/a = not available

## *Figure Legends*

Figure 1. Available levels of potassium and calcium for semisolid (10 days) and liquid media (2 days). Calculated levels are based on media formulation (Table 1) and have been adjusted to reflect 4.5% volume loss during autoclaving. Gel refers to Gelrite. Liquid control media lacked activated carbon but were otherwise identical in composition to liquid media with activated carbon. T1 and N1 designate tissue culture-grade activated carbon (Sigma C-9157) and untreated activated carbon (Sigma C-5260), respectively. Error bars depict the 95% confidence interval based on three or more replications.

Figure 2. Available sodium for semisolid (10 days) and liquid media (2 days). See also Figure 1 legend.

Figure 3. Available magnesium for semisolid (10 days) and liquid media (2 days). See also Figure 1 legend.

Figure 4. Available manganese and iron for semisolid (10 days) and liquid media (2 days). See also Figure 1 legend.

Figure 5. Available zinc and boron for semisolid (10 days) and liquid media (2 days). See also Figure 1 legend. Excess copper (20x) and zinc (2x)) were added to liquid media to compensate for adsorbtion by AC. Liquid control media lacked activated carbon but were otherwise identical in composition to liquid media with activated carbon and included elevated levels of zinc and copper.

Figure 6. Available copper for semisolid (10 days) and liquid media (2 days). See also Figure 1 legend. Excess copper (20x) and zinc (2x)) were added to liquid media to compensate for adsorbtion by AC. Liquid control media lacked activated carbon but were otherwise identical in composition to liquid media with activated carbon and included elevated levels of zinc and copper.

Figure 7. Available copper for nonautoclaved and autoclaved liquid media vs. time (no data were available at 12 days for the nonautoclave treatment). Control media lacked activated carbon but were otherwise identical in initial composition to liquid media with activated carbon and included elevated levels of zinc and copper. Activated carbon was tissue culture-grade (Sigma C-9157, designated T1). Error bars depict the 95% confidence interval based on three or more replications.

Figure 8. Available element levels (Fe, Cu, Zn, Mn) after 2 days for different liquid media with activated carbon (T1) are compared. Treatment labelled "No Organics" refers to media formulated with mineral nutrients only including Fe-EDTA. Treatment labelled "No Fe-EDTA" refers to media formulated with mineral nutrients only, without Fe-EDTA.

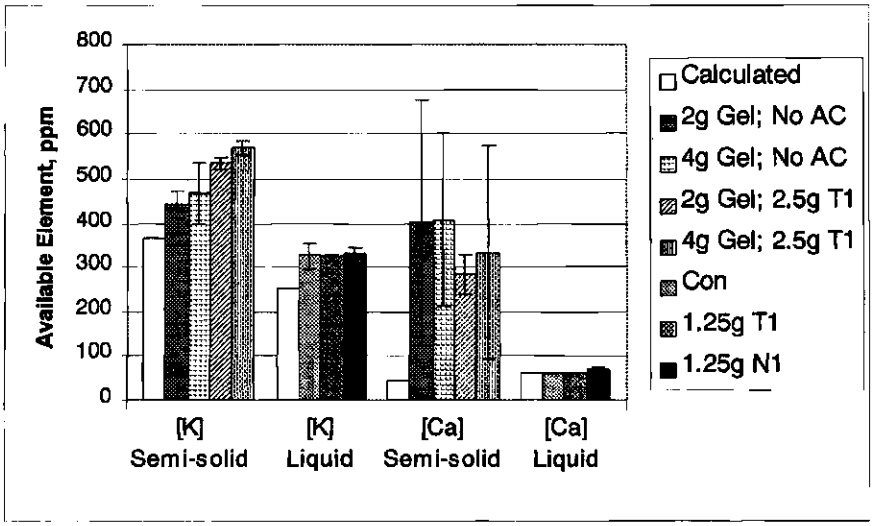


Figure 1.

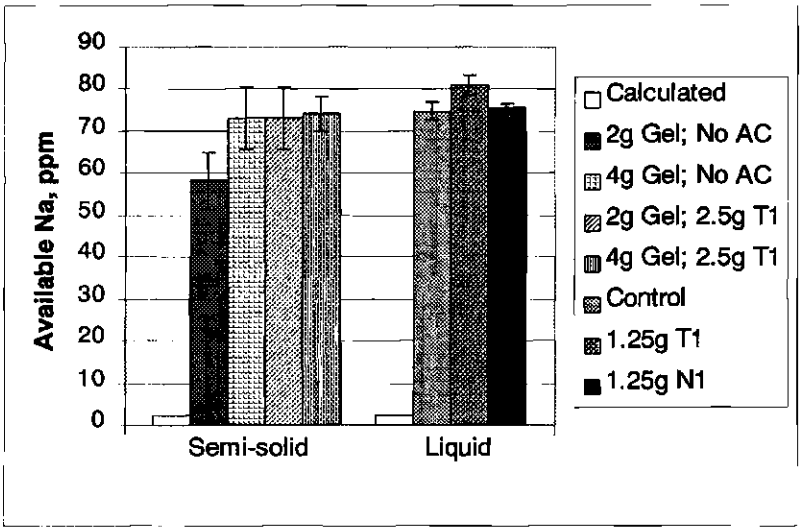


Figure 2.

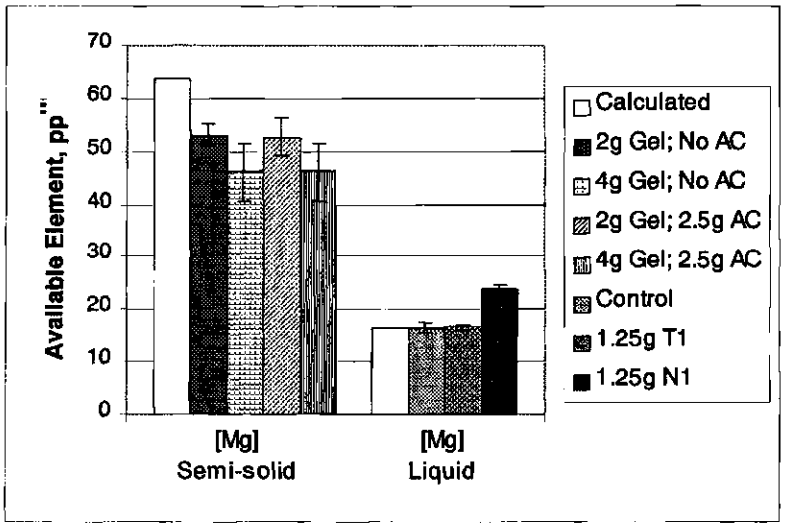


Figure 3.

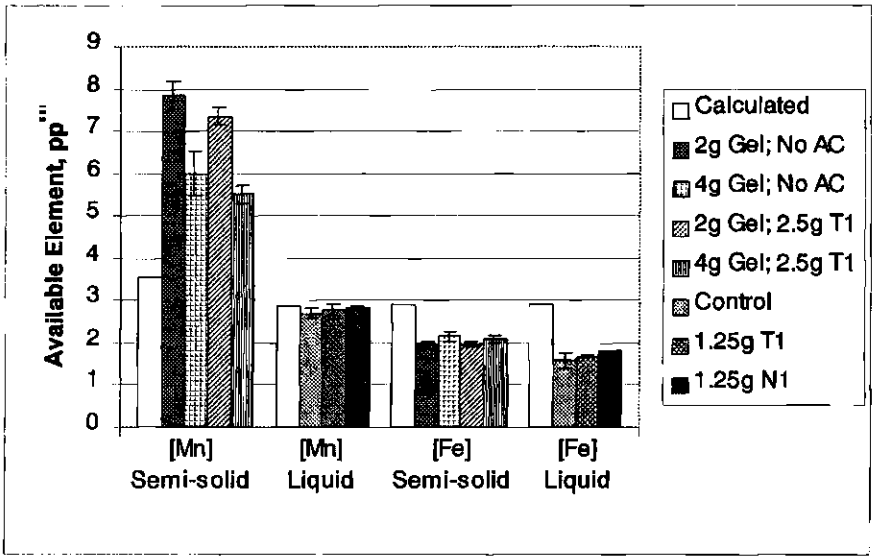


Figure 4.

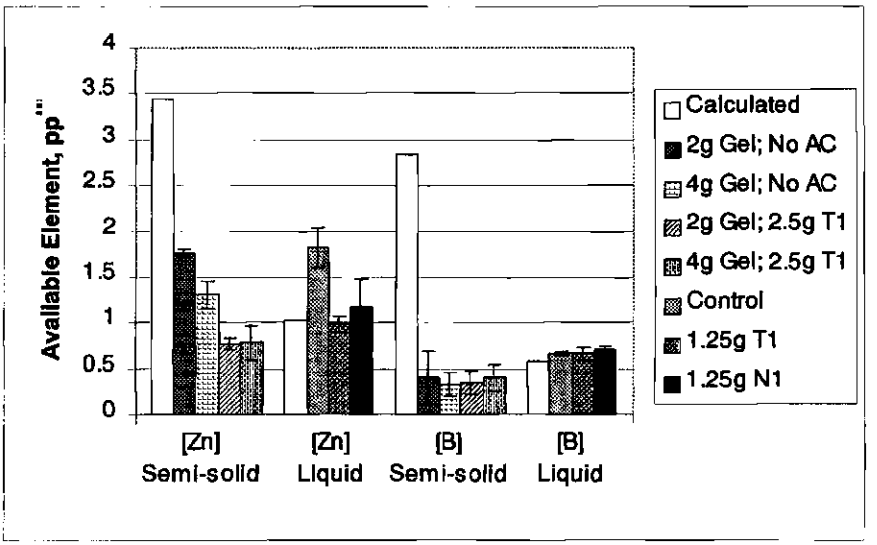


Figure 5.

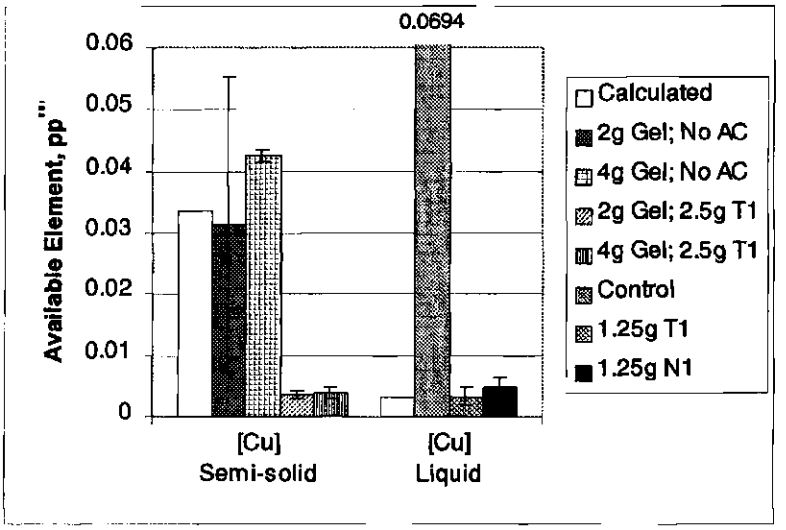


Figure 6.

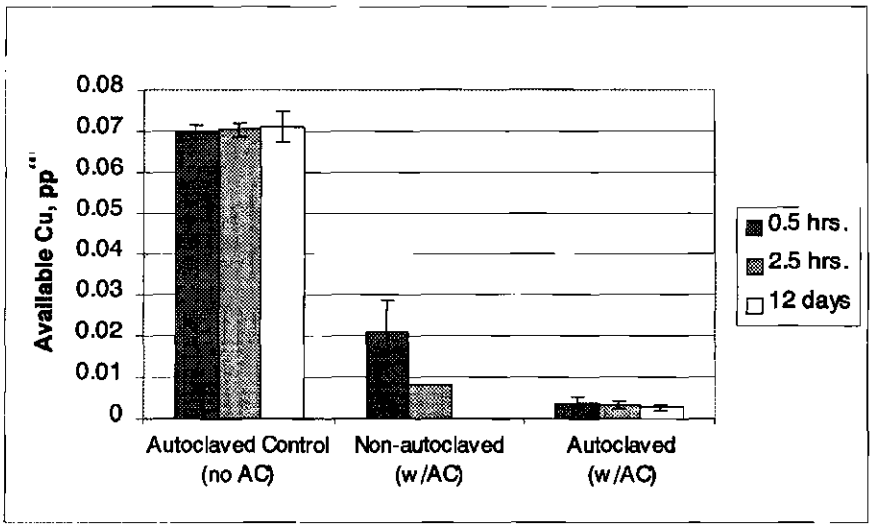


Figure 7.

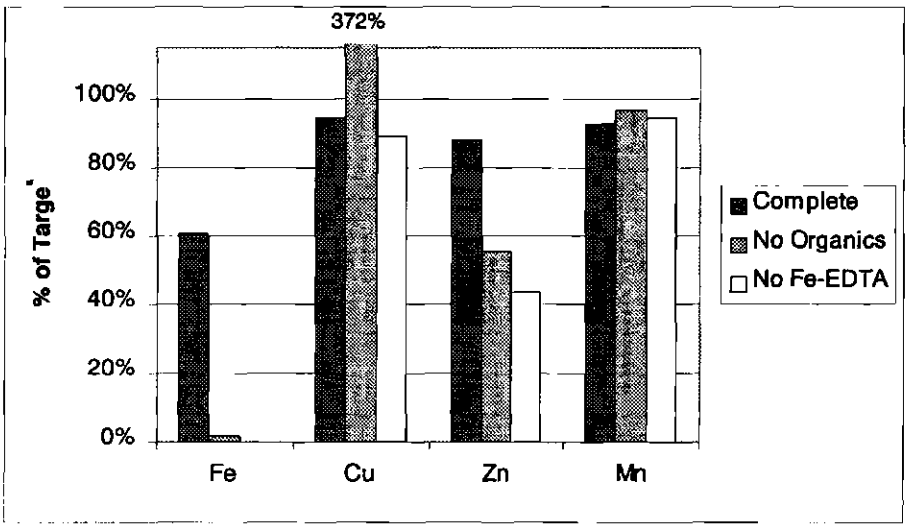


Figure 8.